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Practical Chromatography

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For nearly thirty years after its discovery by Tswett (*1*) in 1906, the technic of chromatographic adsorption remained neglected; it was revived in the early 1930's by Kuhn and by Karrer for their work on the carotenoids. However, the method has unfortunately become associated in the mind of the average chemist with the laborious isolation of minute amounts of such compounds as carotenoids and vitamins. It is not generally realized that quantities up to several hundred grams of many common organic reaction mixtures may thus be separated in laboratory-scale equipment. A full discussion of the procedure may be found in the two books (*2,3*) now available in English; some of the methods favored in our laboratory will be presented here.

Chromatography is the art of separating substances by passing them in solution through a deep layer of adsorbent material generally contained in a vertical glass tube. The components of the mixture will possess varying affinities for the adsorbent and will be concentrated in a series of clearly defined zones. At first, these zones will be grouped closely at the top of the column, but continued passage of pure solvent will cause them to "develop," i.e., to separate, broaden, and move downward. They may now be separated, for instance, by washing the lowermost zones completely through the tube into the filtrate ("liquid" chromatogram) or by mechanically extruding the cylinder of adsorbent, cutting the zones apart and extracting the adsorbed material with a more polar solvent. This process is called "elution."

The adsorptive affinity of a compound for a given adsorbent depends chiefly on its structure; the more polar it is, the more strongly will it be adsorbed. The common functional groups, in approximate order of decreasing polarity, are carboxyl, hydroxyl, nitro, azo, carbonyl, amino, carbalkoxy, and phenyl. The presence of conjugated systems considerably augments the adsorptive affinity. The adsorbability of compounds with the same functional group increases with the molecular weight. Tars are very strongly adsorbed. Chromatography is usually applicable only to compounds whose molecular weights are greater than 125. If this condition is fulfilled, and the molecule contains at least one carboxyl group or equivalent such as a hydroxyl group, two nitro or azo groups, three fused benzene rings, or four conjugated double bonds, the method may well be of value.

A general rule may also be formulated for solvents; the more polar a solvent, the better eluting agent it is. The commonly used solvents, in order of increasing polarity, are petroleum ether, ben-

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Adsorbent	*Source*	*Activity*	*Rate of Flow*
Alumina (Brockmann)	Merck and Co., Inc. Rahway, N. J.	strong	fast
Magnesia	Westvaco Chlorine Products Corp. New York, N. Y.	medium	slow
Hydralo	J. T. Baker Chemical Co. North Phillipsburg, N. J.	very strong	medium
Tricalcium phosphate	General Chemical Co. New York, N. Y.	medium	slow
Doucil	American Doucil Co. Philadelphia, Pa.	weak	very fast

zene, ether, methanol, water, and acetic acid. Mixtures are usually chromatographed from the first two, the first choice being petroleum ether, and eluted by the last four. If poor solubility precludes the use of petroleum ether, the least polar solvent possible should be chosen. Frequently, development is hastened by increasing the polarity of the solvent slightly. Common elution formulae are ether containing 2-5% water and 20-25% methanol or acetic acid.

Although a great many materials have been used as adsorbents, those listed in the table will fulfill most needs.

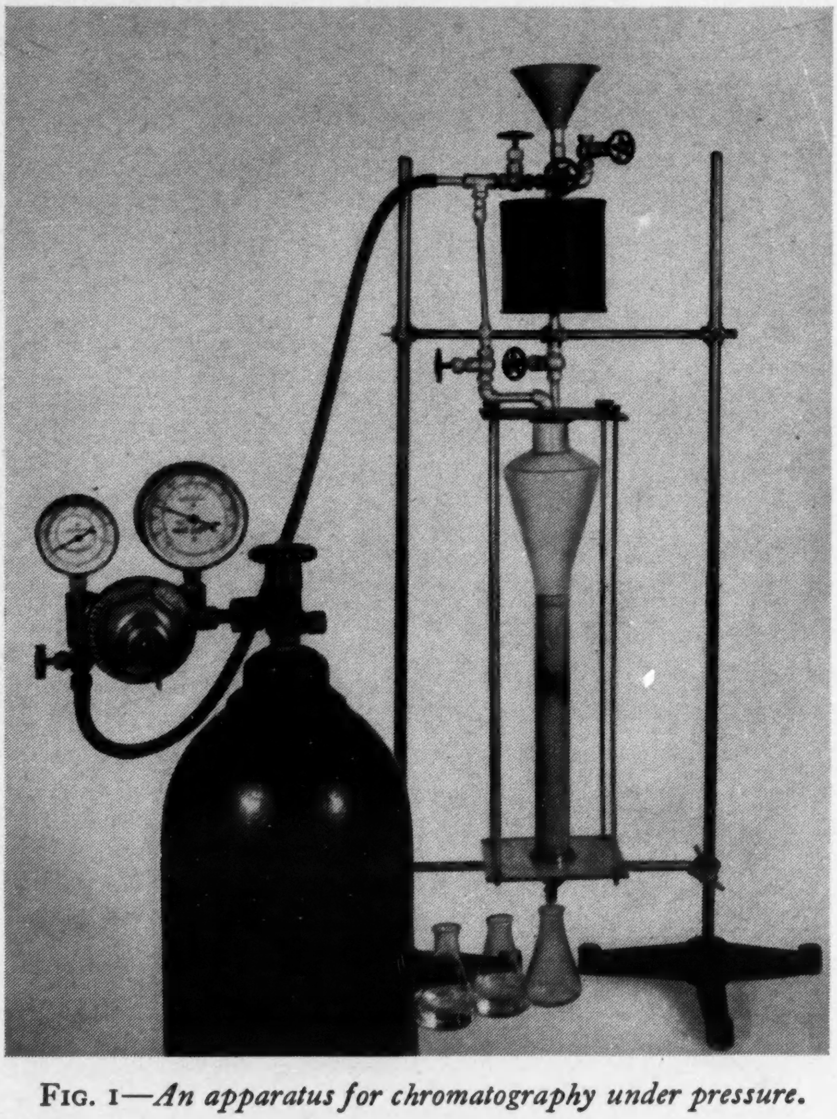


FIG. 1—*An apparatus for chromatography under pressure.*

The choice of adsorbent must be empirical and will be indicated, in the case of new materials, by the results of preliminary micro-experiments. The number of these may be limited by the following consideration. If the mixture contains but few components of quite different adsorbabilities, the liquid chromatogram technic can probably be used. Then only the fast-flowing adsorbents are to be considered, as this technic is not applicable to the slow-flowing adsorbents, unless a pressure column (Fig. 1) (*4*) is used. Conversely, if a complex, difficultly separable mixture is in hand, the greater delineating powers of the slow-flowing adsorbents (which are always admixed with 30% by weight of Celite [Johns-Manville Sales Corp.]) are desirable.

Too strong an adsorbent must not be used; development, or even elution, may be

impossible. Care must also be taken that the chemical nature of the adsorbent does not destroy or alter the material. In particular, acids must not be adsorbed on basic adsorbents. In general, the Brockmann type of alumina should be tried first, followed by magnesia, if a slow-flowing adsorbent is indicated, or Doucil, if the alumina proves too strong.

That most organic compounds are colorless need not be a deterrent to the use of chromatographic adsorption; four practical methods for the location of colorless compounds are available. The simplest is empirical application of the liquid chromatogram technic; a succession of increasingly polar solvents, such as petroleum ether-benzene-ether mixtures, are passed through the column and collected separately. This method will succeed only if the substances have quite different adsorbabilities.

The second method consists in applying to the extruded column a chromogenic reagent, such as tetranitromethane for unsaturated compounds, ferric chloride for phenols, picric acid for amines, etc. The column is then cut according to the zones revealed.

The third, and most striking, method is to observe the fluorescence produced in the adsorbed compounds by ultraviolet light. A considerable proportion of chromatographable compounds will fluoresce, and the method is of wide applicability. No special column constructions are necessary, and a small, portable ultraviolet lamp is very satisfactory.

Lastly, prior to chromatography, the colorless compounds may be transformed to colored ones by esterification with p-phenylazobenzoyl chloride, or by the formation of dinitrophenylhydrazones. The former reagent has been found to be of particular value.

The practice of chromatography is best illustrated by a concrete example. The apparatus used is shown in Fig. 2. The dimensions of the tube are 3 x 50 cm., or a length equal to 15-20 times the diameter. The column was prepared and used in the following manner: A plug of glass wool was inserted in the column to support the adsorbent. Then the full vacuum of a water aspirator was applied to the column, and the adsorbent (alumina) introduced in small portions until the column was one-quarter full. The whole assembly was then pounded vigorously on the workbench fifteen or twenty times to pack the powder into place, the next quarter added, the pounding process repeated, and so forth.

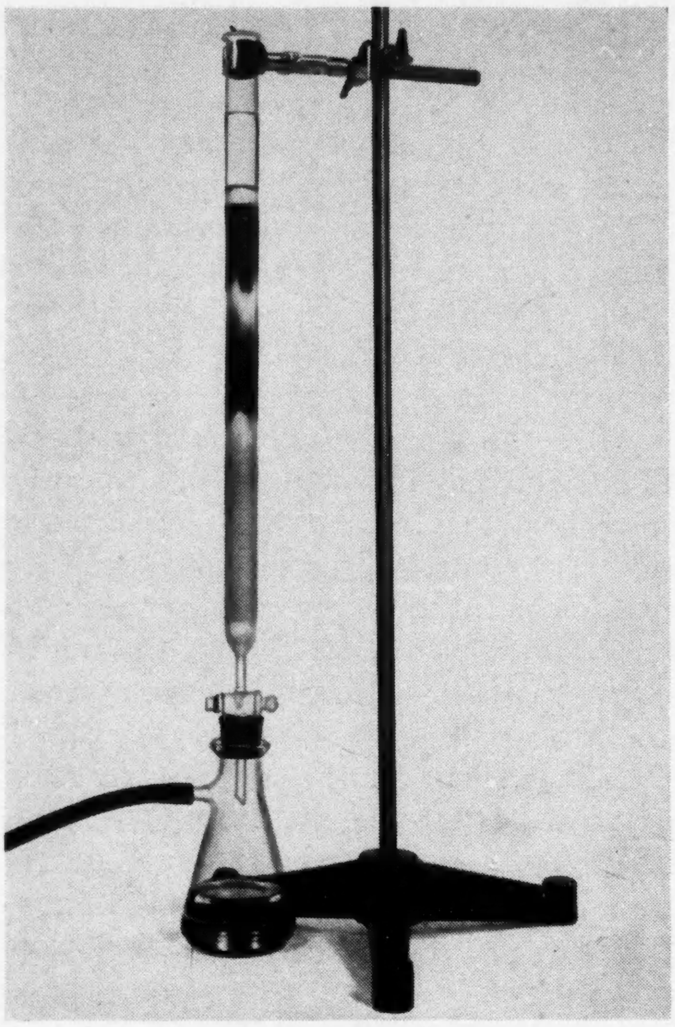


FIG. 2—*Chromatographic separation of p-aminoazobenzene and p-hydroxyazobenzene, using suction.*

Benzene was introduced, the vacuum adjusted by the stopcock to give a slow rate of flow, and a solution of 2 g. p-aminoazobenzene and 2 g. p-hydroxyazobenzene in 100 ml. benzene (the minimum necessary) added. (For photographic purposes the column was underloaded by 50%.) When this had been nearly adsorbed, passage of fresh benzene was started. The originally con-

tiguous orange (upper) and yellow zones of the hydroxy and amino compounds, respectively, moved apart, and a small discolored zone of impurities appeared at the very top (Fig. 2). The polarity of the developer liquid was increased by using 1:1 benzene-ether, and the lower zone washed through into the filtrate (liquid chromatogram), the upper zone remaining fixed.

After the amino compound had been thus removed, the column was allowed to go dry. (It is of the utmost importance that this should not occur until completion of the chromatogram.) The tube was removed from the flask, placed horizontally on the workbench over paper, and the cylinder of adsorbent extruded by the application of a gentle stream of air to the bottom of the tube. The zone of the hydroxy compound was separated from the uppermost small zone of impurities and the lower empty spaces, added to an elution mixture of ether containing 20% acetic acid and 5% water, and filtered through a layer of Celite on a Buchner funnel. The total time required was two hours.

Chromatography has been applied to the following classes of compounds: Dyes of various types; aromatic nitro and amino compounds; phenols and their esters and ethers; homologs of naphthalene; polyenes; sterols; hormones; vitamins; enzymes; terpenes; and alkaloids. The list is not complete but indicates the wide applicability of the method, which is often capable of supplementing, or supplanting, fractional distillation or crystallization.

References

(*1*) Tswett, *Ber. Deut. Bot. Ges.* **24**, 235 (1906)

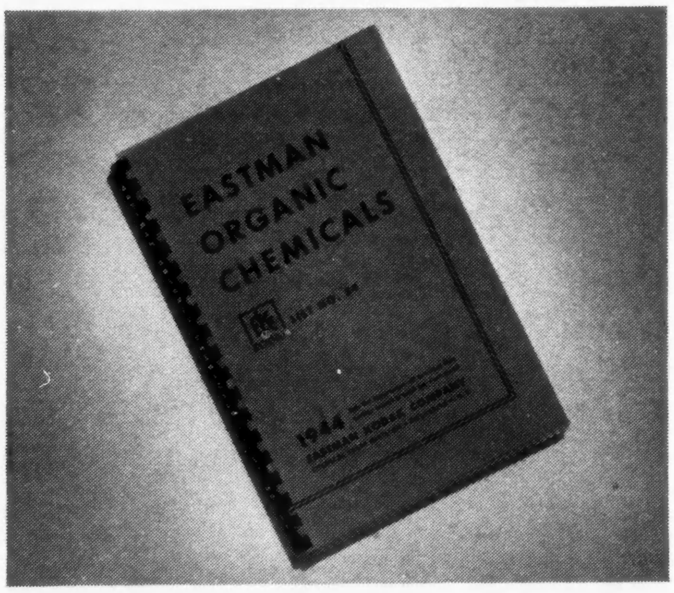
(*2*) Strain, Chromatographic Adsorption Analysis, *Interscience Publishers, Inc.*, New York (1942)

(*3*) Zechmeister and Cholnoky, Principles and Practice of Chromatography, *John Wiley and Sons*, New York (1941)

(*4*) Potts and Koch, *Proc. Soc. Exptl. Biol. Med.* 37, 300 (1937)

A New List of Eastman Organic Chemicals

THE thirty-fourth edition of the *Catalog and Price List of Eastman Organic Chemicals* not only lists more than 3000 synthetic organic chemicals but, in addition, gives their structural formulas. This feature, a radical departure from previous editions, greatly increases the importance and value of the new catalog. The format has been changed to the larger, more convenient, standard 6½ x 9½-inch size, and the typographic design has been modernized. As in the previous edition, the main catalog section is supplemented by nine convenient lists of special reagents, indicators, vitamins, etc.



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Errata

Vol. 16, No. 2, p. 1 (1944)

IN Fig. 1 of the article on "Synthetic Antimalarials" by C. V. Wilson, a chlorine atom was inadvertently omitted from position 6 in the formula for Atabrine. In the preceding formula of the acridine derivative, the H in the 9 position should be replaced by Cl.